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PROTEIN POLYMORPHISMS IN THE HARD CLAMS *MERCENARIA MERCENARIA* AND *MERCENARIA CAMPECHIENSIS*

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The northern hard clam, *Mercenaria mercenaria*, is found intertidally and in shallow waters from the Gulf of St. Lawrence to the Gulf of Mexico. The southern hard clam, *M. campechiensis*, ranges from New Jersey to the Gulf of Mexico and the West Indies (Menzel, 1969). It is found offshore in the northern half of its range and both inshore and offshore in the southern half. Adults of both species are non-mobile and long-lived, surviving up to 20 years. They lie slightly buried in sandy or muddy bottoms and filter the overlying water to obtain food. These bivalves are highly prolific and their larvae are broadcast over wide areas. An average female may produce 20 to 30 million eggs during a summer (Ansell, 1967; Davis and Chanley, 1956). The eggs and sperm are shed into the overlying water where fertilization occurs. Larvae develop in the water column for a period of a week or more (Carriker, 1961). During this time they may be dispersed great distances by water movements. Ample opportunity exists for genetic exchange among adjacent populations.

This paper reports preliminary observations on the population genetics of these important representatives of the estuarine benthic community. Data were obtained by studying the electrophoretically resolvable forms of the hard clam's enzymes. The use of isozymes to answer genetic questions was popularized by Hubby and Lewontin (1966). The rationale for this approach was advanced by Shaw (1965), Gooch and Schopf (1970) and others. Briefly, since the electrophoretic mobility of an enzyme is determined by its primary structure, any enzyme variants detected by electrophoresis are equated with alleles at the locus controlling the primary structure of that enzyme. Hence, with this technique, phenotypic differences reflect genotypic differences at single loci. Such measures of genetic variation may be used to explore a host of previously unaswerable questions.

The questions posed in this study were: (1) how much variation exists within the gene pool of *M. mercenaria* and *M. campechiensis* populations, (2) what genetic differences exist among widely separated populations of *M. mercenaria* and *M. campechiensis*, and (3) does the gene pool of *M. mercenaria* differ greatly from that of its southern congener, *M. campechiensis*.

Genetic data were obtained for the following enzymes: malate dehydrogenase-NAD form (MDH); and two enzymes of unknown substrate, tetrazolium oxidase (TO) and an esterase (EST). Lactate dehydrogenase (LDH) data were treated in a previous publication (Pesch, 1972) but some of that information will be included here for discussion.

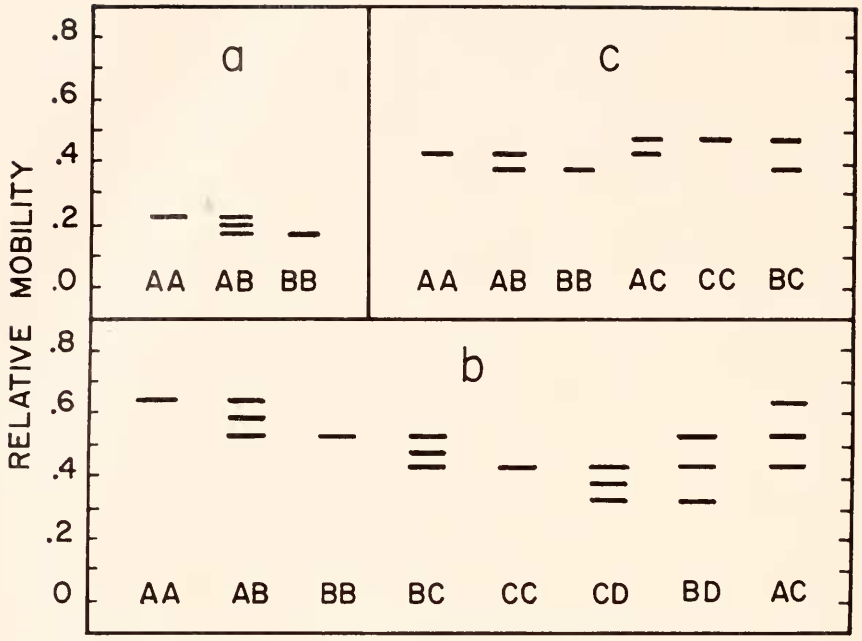

Figure 1. Phenotypes observed in populations of *M. mercenaria* and *M. campechiensis;* (a) NAD—Malate dehydrogenase, (b) tetrazolium oxidase and (c) an esterase. Anode is at top. Band mobilities were measured relative to bromphenol blue dye front.

Materials and Methods

Four populations of *M. mercenaria* were sampled: one each from the Bideford River, Prince Edward Island, Canada; Boothbay Harbor, Maine; Narragansett Bay, Rhode Island; and Wadmalaw Island, South Carolina. For comparison, two populations of *M. campechiensis*, a southern congener, were sampled: one from Shackleford Banks near Beaufort, North Carolina, and one from Tampa Bay on Florida's Gulf Coast. The samples were collected during the summer of 1970 and included animals from 6.2 to 12.7 cm in length with approximately equal proportions of sexes. A minimum of 50 animals from each population was assayed for each of the enzymes. A pooled sample of Rhode Island *M. mercenaria* provided a reference to compare band mobilities of the enzymes among the populations studied. It is assumed that some forms of the enzymes studied are common to both species because of the identical electrophoretic mobilities of these forms. Enzyme patterns were photographed with Kodak Plus X Pan film.

Preparation of tissue extracts

Tissues were homogenized and extracted in a Tris buffered sucrose solution as described by Pesch (1972). Electrophoretic patterns were stable for several months in extracts stored at −20°. Gill, mantle, muscle and whole animal homogenates were tested. The esterase patterns studied were observed in gill tissue

TABLE I

Malate dehydrogenase NAD form (MDH) alleles and phenotypes from four populations of Mercenaria mercenaria *and two populations of* Mercenaria campechiensis

Phenotypic frequencies observed (expected)

Genotypes	*M. mercenaria*				*M. campechiensis*	
	Can.	Me.	R. I.	S. C.	N. C.	Fla.
AA*	50 (50.0)	47 (46.1)	58 (58.0)	55 (55.0)	58 (58.0)	52 (52.0)
BB		1 (0.1)		0 (0)		
AB		2 (3.8)		1 (0.9)		
	50	50	58	56	58	52
Allelic frequencies						
A	1.00	0.96	1.00	0.99	1.00	1.00
B		0.04		0.01		

* Alleles are arbitrarily labelled with letters.

only; the other enzymes were observed in all tissues tested. Mantle tissue arbitrarily was chosen for the remaining enzyme assays.

Electrophoretic and staining techniques

Disc-polyacrylamide gel electrophoresis was performed on an EC Apparatus vertical cell (model EC 470) as described by Pesch (1972). Buffers for electrophoresis and stains for enzyme demonstration were as follows: *Malate dehydrogenase—NAD form (MDH)*; buffers; same as described by Pesch (1972) for analysis of LDH; stain; nitro blue tetrazolium method modified from Latner and Skillen (1968) by deletion of phenazine methosulfate. *Tetrazolium oxidase (TO)*; buffers; same as for MDH; stain: same as for MDH but with a trace of phenazine methosulfate added. *Esterase (EST)*; buffers; stacking gel buffer same as for MDH, running gel buffer 0.124 M Tris adjusted to pH 7.8 with HCL. Electrode buffer 0.004 M Tris adjusted to pH 8.0 with glycine; Stain; Fast Blue RR salt method with α-naphthyl acetate substrate as described by Latner and Skillen (1968).

RESULTS

Malate dehydrogenase—NAD from (MDH)

Phenotypes for MDH are either one or three bands with no differences between sexes (Fig. 1a). All but four of 324 individuals sampled had a single band of identical electrophoretic mobility (Table I). These were presumably homozygotes for a common allele. One individual had a single band of lesser mobility and was presumably a homozygote for a different allele. Three individuals had both of the above bands plus one of intermediate mobility. These three are thought to be heterozygotes containing both alleles. This enzyme appears to be a dimer with single locus autosomal inheritance.

TABLE II

Tetrazolium oxidase alleles and phenotypes from four populations of Mercenaria mercenaria *and two populations of* Mercenaria campechiensis
Phenotypic frequencies observed (expected)

Genotypes	*M. mercenaria*				*M. campechiensis*	
	Can.	Me.	R. I.	S. C.	N. C.	Fla.
AA	2 (2.4)	18 (15.7)	28 (28.8)	28 (26.6)	1 (1.9)	2 (0.9)
BB	30 (30.4)	12 (9.7)	3 (3.7)	6 (5.0)	14 (12.7)	17 (18.1)
CC			0 (0)	0 (0)	6 (4.8)	2 (3.8)
DD					0 (0)	0 (0)
AB	18 (17.2)	20 (24.6)	22 (20.5)	21 (23.2)	11 (9.9)	6 (8.0)
AC			1 (0.8)	0 (0.8)	7 (6.0)	4 (3.7)
AD					0 (0.4)	0 (0.1)
BC			0 (0.3)	1 (0.3)	12 (15.6)	20 (16.6)
BD					1 (1.0)	1 (0.6)
					1 (0.6)	0 (0.3)
	50	50	54	56	53	52
Allelic frequencies						
A	0.22	0.56	0.73	0.69	0.19	0.13
B	0.78	0.44	0.26	0.30	0.49	0.59
C			0.01	0.01	0.30	0.27
D					0.02	0.01

Tetrazolium oxidase

The metabolic substrate of this enzyme is not known. The oxidase is visualized as light bands on a dark blue tetrazolium background. Patterns consisting of one or three bands are found (Fig. 1b). Single banded patterns are thought to be from homozygotes. Patterns with three bands had two bands with mobilities corresponding to the bands from homozygotes and a third band of intermediate mobility. These are thought to be from heterozygotes. This enzyme is probably a dimer inherited at a single locus with multiple alleles.

A north-south allelic cline paralleling that found for LDH (Pesch, 1972) is suggested for this enzyme (Table II). Two alleles appear in the Canadian population of *M. mercenaria*. The number of alleles increases to four in the Florida population of *M. campechiensis*. In all six populations the phenotypic frequencies fit the Hardy-Weinberg equilibrium model. Only phenotypes with expected frequencies of 10% (5 individuals) or more per population were included in chi-square tests. Tests of significance were made at the $P = 0.05$ level.

Esterase

Esterase enzymes have broad substrate specificities. The assay, with α-naphthyl acetate as substrate, yielded a bewildering array of patterns on electropherograms of gill tissue. However, prominent dark bands of medium mobility consisently were present in patterns explainable by the genetic mechanism of

TABLE III

Esterase (α-napthyl acetate) alleles and phenotypes from four populations of Mercenaria mercenaria *and two populations of* Mercenaria campechiensis
Phenotypic frequencies observed (expected)

Genotypes	*M. mercenaria*				*M. campechiensis*	
	Can.	Me.	R. I.	S. C.	N. C.	Fla.
AA	42 (41.6)	44 (41.5)	40 (37.9)	54 (54.0)	46 (44.7)	43 (42.0)
BB	0 (0)	2 (0.1)			0 (0.1)	2 (1.0)
CC	1 (0.4)	1 (0.2)	3 (0.9)	0 (0)	1 (0.4)	
AB	2 (1.8)	0 (3.6)			3 (3.5)	11 (13.0)
AC	7 (8.1)	4 (5.4)	7 (11.3)	2 (2.0)	6 (8.0)	
BC	0 (0.2)	0 (0.2)			1 (0.3)	
	52	51	50	56	57	56
Allelic frequencies						
A	0.89	0.90	0.87	0.98	0.88	0.87
B	0.02	0.04			0.04	0.13
C	0.09	0.06	0.13	0.02	0.08	

multiple alleles at a single locus. To facilitate interpretation of highly polymorphic electropherograms, only these dark bands were considered.

Individuals had either one or two dark bands (Fig. 1c). Phenotypes with one band were assumed to be homozygotes, those with two bands heterozygotes. All six populations displayed at least two alleles but a single common allele predominanted (Table II). Phenotypic frequencies fit those predicted by the Hardy-Weinberg equilibrium model for each population.

DISCUSSION

Intrapopulation variance

General conclusions about the genome are made with reservation since only four loci were considered. Obviously more loci need to be examined. However, with at least three of four loci polymorphic in all populations examined (Table IV) it seems safe to infer that the level of genetic variability of these species approaches that of other species.

Lewontin and Hubby (1966) in their classic study of *Drosophila pseudoobscura* estimated that 39% of the loci in the genome were polymorphic for the whole species with an average of 30% of all loci polymorphic for any given population. These estimates were based on data for 21 loci. Selander and Yang (1969), using data from 40 loci, estimated levels of genetic polymorphism in a wild population of the house mouse, *Mus musculus*, and obtained results remarkably similar to those of Lewontin and Hubby (1966). Several marine invertebrates have been examined in similar fashion. Selander *et. al.* (1970) studied 25 loci in 64 individual horseshoe crabs (*Limulus polyphemus*) from four localities on the East and Gulf Coasts. They estimated (page 412) that "single populations of *Limulus*

TABLE IV

Number of alleles found at four loci in four populations of Mercenaria mercenaria *and two populations of* Mercenaria campechiensis

	MDH	EST	TO	LDH	Total	Alleles/Locus
M. mercenaria						
Can.	1	3	2	2	8	2.00
Me.	2	3	2	4	11	2.75
R. I.	1	2	3	5	11	2.75
S. C.	2	2	3	5	12	3.00
M. campechiensis						
N. C.	1	3	4	6	14	3.50
Fla.	1	2	4	7	14	3.50

are, on the average, polymorphic at 25.0% of their loci." Gooch and Schopf (1970) report genetic variability in two species of ectroprocts found in the vicinity of Woods Hole, Massachusetts. *Schizoporella unicornis* had two of eight loci polymorphic (25%) while *Bugula stolonifera* had 6 of 11 loci polymorphic (54.5%).

Both populations of *M. campechiensis* have a total of 14 alleles segregating at four loci for an average of 3.5 alleles per locus. The Canadian, Maine, Rhode Island, and South Carolina populations of M. mercenaria averaged 2.0, 2.75, 2.75, and 3.0 alleles per locus, respectively. These compare with 2.0 alleles per locus for *D. pseudoobscura* (Hubby and Lewontin, 1966) and 2.25 alleles per locus for *M. musculus* (Selander and Yang, 1969).

If the variation contained in the genome of *Mercenaria* does approach the level found in other species, a wide variety of genotypic combinations is potentially possible. The reproductive rate of the hard clam is geared to exploit this potential. An average adult female produces approximately 25 million eggs during a single sqawning season (Davis and Chanley, 1956). High fecundity permits re-shuffling of genetic material within a population. Dispersal, via planktonic larvae, permits maintenance of variation by genetic exchange among populations.

Interpopulation variance

The sampling transect extended from Prince Edward Island, Canada to Tampa Bay, Florida. Over this range, three patterns were notable in the data (Table IV). Monomorphism is approached by the MDH locus with a single allele perdominating in all populations. Polymorphism is pronounced at the EST locus but the data show no patterned shift. Clines were observed at both the TO and the LDH loci.

Gooch, Smith and Knupp (1972) conducted a similar survey on the estuarine mud snail *Nassarius obsoletus*. Their sampling transect extended from Cape Cod, Massachusetts to Beaufort, North Carolina, which overlaps the middle third of the present survey. Six loci were examined in the mud snail; four were monomorphic; two were polymorphic. Gene frequencies were uniform along the transect. Interestingly, three enzymes (MDH, TO, and LDH) were examined in both surveys. In the mud snail MDH was monomorphic, but with an aberrant band present in 1 to 2 per cent of the individuals. The patterns for the hard clam MDH are similar,

however the low frequency aberrant bands are identifiable as second alleles. Tetrazolium oxidase of the mud snail was present consistently in two forms. The authors regarded these as representing separate loci monomorphic for single alleles. In the hard clam TO is controlled at a single locus and is clearly polymorphic, displaying a latitudinal cline. Lactate dehydrogenase was found to be polymorphic in both studies. In the mud snail three alleles were found in uniform frequencies along the transect. In the hard clam LDH displayed a marked latitudinal cline.

Both the mud snail and the hard clam are physiologically tough, surviving in a diversity of environments over broad geographic ranges. The mud snail presents a uniform genetic picture while the hard clam presents a varied array of genetic patterns. It seems they have followed separate genetic routes to achieve durability. Lewontin (1957) suggests two routes a population may take to survive a change in environment. The first is individual homeostasis. A population survives because each of its members is able to survive in a variety of environments. Heterozygosis is thought to be the genetic basis of such homeostasis. Populational homeostasis is the second route to survival. The genetic composition of a population is diverse, containing an array of highly varied genotypes. Each genotype would have a distinct range of environmental limits so that the sum of a variety of genotypes would have a greater range of survival than any single genotype. At least some of these genotypes would be able to survive even drastic environmental changes.

The mud snail presents a uniform genetic picture, suggesting that this species survives by the route of individual homeostasis. The individual is "optimized." The hard clam presents a varied genetic picture suggesting that they survive by the route of populational homeostasis. A diverse genetic composition exploited by high fecundity produces an almost infinite array of genotypes.

Gooch *et. al.* (1972) ascribe the genetic uniformity of the mud snail to either mechanisms of balancing selection, pervasive gene flow or a combination of these. They find it difficult to accept the mechanism of balancing selection because of the varied environments represented by their samples. Instead they argue in favor of extensive gene flow, citing the vector of planktonic larvae. However, this vector is common also to the hard clam.

A study of direct relevance was reported by Levinton (1973). He tested the role of environmental variability in regulating genetic variability. Six bivalve mollusk species were selected from (page 75) "an inferred gradient of environmental variability." Estimates of genetic variability were based on polymorphisms observed in two enzymes, phosphohexose isomerase (PHI) and leucine aminopeptidase (LAP). He concluded (page 76) "it seems clear that species supposed to be experiencing more environmental variability are more polymorphic than those living in more constant environments." Two explanations were considered. One is selection for heterozygotes in a varying environment. The second is diversifying selection in a heterogeneous environment with extensive gene flow.

Levinton (1973) champions the view that spatial and temporal environmental variances selectively enhance the amount of genetic variation in a population. A second view holds that selectively neutral alleles are important (Kimura, 1968; King and Jukes, 1969). Pertinent to this view is a study reported by Schopf & Gooch (1971). A sampling of deep-sea invertebrates, representing a very stable environment, revealed a high level of genetic variability. These data are most easily explained by assuming the presence of selectively neutral alleles.

Table V

Percentage of heterozygotic genotypes found at four loci in four populations of Mercenaria mercenaria *and two populations of* Mercenaria campechiensis

	MDH	EST	TO	LDH	Mean
M. mercenaria					
Can.	0	17.3	36.0	100	38.33
Me.	4.0	7.8	40.0	86.0	34.45
R. I.	0	14.0	42.6	83.6	35.05
S. C.	1.8	3.6	41.1	82.7	32.30
M. campechiensis					
N. C.	0	17.5	60.4	41.5	29.85
Fla.	0	19.6	59.6	70.6	37.45
Mean	0.97	13.30	46.62	77.40	34.57

The hard clam data are not so easily explained. The presence of clines at two of four loci (TO, LDH) suggests adaptive significance. The high number of alleles in the southern populations may arise from gene flow within a spatially heterogeneous environment or from the presence of neutral alleles surviving in a relatively more benign, stable environment. Either source is a possibility. Contrasting data exist on the occurrence of heterozygotes (Table V). The loci for MDH and EST show no trends. Tetrazolium oxidase is characterized by an increasing number of heterozygotes in the southern populations. This is an expected consequence of an increased number of alleles. Lactate dehydrogenase has a maximum of heterozygotes in the Canadian population. This trend may reflect increased selection for heterozygotes at this locus by fluctuating environments. The total picture presented by the hard clam data suggests a complex of selective processes probably of differing biological significance.

Species comparison

Mercenaria mercenaria, the northern hard clam, and *Mercenaria campechiensis*, the southern hard clam, along with several subspecies, occur along the Atlantic and Gulf Coasts of North America (Menzel, 1969). *M. mercenaria* is confined to inshore waters from Canada to the Gulf of Mexico. *M. campechiensis* occurs offshore in the northern part of its range, inshore south of Cape Kennedy, and in both habitats in the Gulf of Mexico.

Menzel (1969) concluded that the subspecies *M. mercenaria notata* and *M. mercenaria alba* had no validity, but suggests that the subspecies *M. mercenaria texana* is a naturally occurring hybrid between the two species. *M. mercenaria* and *M. campechiensis* hybridize readily in the lab and have been reared through the F_2 generation. "Shell morphology of about $\frac{3}{4}$ of the F_2's is very similar to the subspecies *M. mercenaria texana* . . ." (Menzel, 1969, page 8).

The ease of raising hybrids in the lab and the presence of intermediates in the field suggest that gene flow may occur between these species. Menzel and Menzel (1965) studied the chromosome complements of both species and their hybrids. They conclude (page 187) that the "homology and regular behavior of the

TABLE VI

Allelic overlap (%) at four loci among four populations of Mercenaria mercenaria *and two populations of* Mercenaria campechiensis

	M. campechiensis		*M. mercenaria*		
	Fla.	N. C.	S. C.	R. I.	Me.
M. mercenaria					
Can.	47	57	54	58	73
Me.	56	67	77	69	
R. I.	67	79	92		
S. C.	63	73			
M. campechiensis					
N. C.	87				

chromosomes of the two species, revealed at meiosis in F_1 hybrid, demonstrate that there is no gross chromosomal barrier to such gene interchange."

In the present study the gene pools of these species are seen to have many alleles in common (Table VI). Of a total 16 alleles observed at four loci in four populations of *M. mercenaria* and two populations of *M. campechiensis*, 12 alleles (75%) are found in both species. Indeed the genetic differences among populations of *M. mercenaria* are greater than differences between the species. For example, comparison of the Canadian and South Carolina populations of *M. mercenaria* revealed that from a total of 13 alleles they shared 7 (54%) in common. The accumulated evidence suggests that *M. mercenaria* and *M. campechiensis* probably represent what Dobzhansky (1970) termed "incipient species." These two species, recently derived from an ancestral line, have not yet reached reproductive isolation.

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SUMMARY

Four gene loci were characterized by polyacrylamide disc-gel electrophoresis of four enzymes in the hard clams, *M. mercenaria* and *M. campechiensis*. Four populations of *M. mercenaria* and two populations of *M. campechiensis* were se-

lected from a transect between Prince Edward Island, Canada and Tampa Bay, Florida. Three of the four enzymes were polymorphic in all six populations. *M. mercenaria* averaged 2.6 alleles per locus for four populations while *M. campechiensis* averaged 3.5 alleles per locus for two populations. Malate dehydrogenase—NAD form (MDH) had a single allele predominating in all populations. A second rare allele appeared in the Maine and South Carolina populations of *M. mercenaria*. Polymorphism was pronounced at an esterase (EST) locus but the data showed no patterned shift along the transect. North to south clines were observed at both the tetrazolium oxidase (TO) and the lactate dehydrogenase (LDH) loci. The different patterns observed for each locus suggest a complex of selective processes probably of differing adaptive significance. In the portion of the gene pools studied these species were found to have many alleles in common (12 of 16). Commonality of alleles, homology of chromosomes, ease of hybridization, and intergrades in the field suggest that these species have not yet reached reproductive isolation.

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